Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (Currently Amended) A method for protecting a mature T cell from cell death, comprising contacting the T cell *ex vivo* with at least two agents selected from the group consisting of an anti-CD28 antibody, a submitogenic amount of an anti-CD3 antibody, an anti-CD2 antibody, a CD28 ligand, interleukin-2 (IL-2), ionomycin, A23187, phorbol-12, 13-dibutyrate, a lectin and a superantigen, wherein the agent increases ~~which augments~~ BCL-X_L protein level in the T cell such that the T cell is protected from cell death.

Claims 2-31. (Canceled)

Claim 32. (Previously Presented) The method of claim 1, wherein said anti-CD3 antibody is OKT3.

Claim 33. (Previously Presented) The method of claim 1, wherein said anti-CD2 antibody is selected from the group consisting of T11.1, T11.2 and T11.3.

Claim 34. (Previously Presented) The method of claim 1, wherein said CD28 ligand is selected from the group consisting of a B7-1 molecule, fragments thereof or modifications thereof and a B7-2 molecule, fragments thereof or modifications thereof.

Claim 35. (Previously Presented) The method of claim 1, wherein said lectin is selected from the group consisting of phytohemagglutinin (PHA), concanavalin (ConA) and pokeweed antigen (PWA).

Claim 36. (Previously Presented) The method of claim 1, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins A, B, C, D and E.

Claim 37. (Previously Presented) The method of claim 1, wherein said T cell is infected with Human Immunodeficiency Virus (HIV).

Claim 38. (Previously Presented) The method of claim 1, wherein said T cell is a mammalian T cell.

Claim 39. (Currently Amended) A method for protecting a mature T cell from cell death in a subject by increasing [augmenting] the level of BCL-X_L protein in said T cell, comprising obtaining said T cell from said subject, contacting said T cell *ex vivo* with at least two agents selected from the group consisting of an anti-CD28 antibody, a submitogenic amount of an anti-CD3 antibody, an anti-CD2 antibody, a CD28 ligand, interleukin-2 (IL-2), ionomycin, A23187, phorbol-12, 13-dibutyrate, a lectin and a superantigen, and reintroducing said T cell into said subject, such that T cell death is inhibited in said T cell of said subject.

Claim 40. (Previously Presented) The method of claim 39, wherein said anti-CD3 antibody is OKT3.

Claim 41. (Previously Presented) The method of claim 39, wherein said anti-CD2 antibody is selected from the group consisting of T11.1, Tb11.2 and T11.3.

Claim 42. (Previously Presented) The method of claim 39, wherein said CD28 ligand is selected from the group consisting of a B7-1 molecule, fragments thereof or modifications thereof and a B7-2 molecule, fragments thereof or modifications thereof.

Claim 43. (Previously Presented) The method of claim 39, wherein said lectin is selected from the group consisting of phytohemagglutinin (PHA), concanavalin (ConA) and pokeweed antigen (PWA).

Claim 44. (Previously Presented) The method of claim 39, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins A, B, C, D and E.

Claim 45. (Previously Presented) The method of claim 39, wherein said T cell is infected with Human Immunodeficiency Virus (HIV).

Claim 46. (Previously Presented) The method of claim 39, wherein said subject is a human.

REMARKS

Applicants thank the Examiner for the telephone conversation of August 13, 2003. As discussed with the Examiner, a Request for Continued Examination of the application is filed herewith.

Claims 1 and 32-46 were pending in the application. Claims 1 and 39 have been amended to more fully and distinctly claim the present invention. Accordingly, claims 1 and 32-46 are currently pending in the present application. No new matter has been added. Any amendments to and/or cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Support for the amendment to claims 1 and 39 to specify that the level of BCL-X_L protein is increased in the T cell is found, for example, at least at page 3, line 25-26 of the specification. Support for the amendment to claims 1 and 39 to recite a "mature" T cell is found in the specification, for example, at least on page 6, lines 29-31, and also by example, in Examples 1-5, which describe utilization of mature T cells.

Specifically, support for the recitation of "mature T cells" is found on page 6, lines 29-31 where it is stated:

> The method of the invention involves "protecting a T cell from cell death". The term "T cell" is art-recognized and is intended to include thymocytes, immature T lymphocytes, ***mature T lymphocytes***, resting T lymphocytes, or activated T lymphocytes.

Also in Example 1, page 33, lines 28-30, the specification details the use of ***mature T cells***:

> To study the effects of T cell activation pathways on T cell survival, ***resting T cells were isolated from human peripheral blood*** by negative selection as previously described (June, C.H. et al. (1987) *Mol. Cell. Biol.* 7:4472-4481).

Within the art, a ***resting cell*** is one that is quiescent, or not undergoing mitosis. In addition, a mature cell is formed at the end of a well-defined developmental program in which a pluripotent stem cell gives rise to a committed progenitor cell and finally to a terminally differentiated cell, *e.g.*, a mature T cell, ***all within the thymus***. Once a T cell becomes terminally differentiated, *e.g.*, mature, it will no longer divide and either circulates in the bloodstream or migrates in the bloodstream from the thymus to a secondary lymphoid organ, *e.g.*, lymph node, spleen, *etc*. Since the Examples cite ***peripheral blood*** as the source of the T cells, they are, by definition, ***mature*** T cells.

Support for the amendment to claims 1 and 39 to recite "a submitogenic amount of an anti-CD3 antibody", is found, for example, at least in Example 1, which provides that submitogenic amounts of anti-CD3 antibody were contacted with T cells in the described experiments (discussed further below), and also in Example 8, at page 43, line 17-18 of the specification, which describes experiments in which T cells were contacted with submitogenic amounts of anti-CD3 antibody.

Specifically, Example 1, page 33, lines 35-39, recites specific amounts of antibody used in the Examples to stimulate crosslinking:

> CD28+ T cells were cultured in medium alone or stimulated by crosslinking the TCR/CD3 complex in the presence or absence of costimulation provided by a CD28-specific monoclonal antibody for 12 hours. Crosslinking of the T cell receptor was performed using plate-immobilized anti-CD3 (G19.4 [at 1 μg/ml]) and costimulation of the T cells was performed with soluble antibody to CD28 (monoclonal antibody (mAb) 9.3) at 1 μg/ml.

Example 8, page 43, lines 17-18 teaches:

> Furthermore, at ***submitogenic doses of anti-CD3***, the induction of bcl-X_L expression is almost completely dependent on CD28 costimulation.

On page 34, lines 27-33, the specification teaches that stimulated cells were not subsequently dividing, since cell counts remained constant and the cells were arrested in late G_1 or G_2 of the cell cycle:

> The maintenance of cell viability in anti-CD3 stimulated and anti-CD3 + anti-CD28 stimulated cells was ***not the result of subsequent T cell proliferation as cell counts done in parallel to the viability assays revealed that the absolute cell number did not change.*** Furthermore, all the cells in the activated populations were ***arrested within the cell cycle at either late G_1 or G_2.*** Cell death in all three populations followed a classic apoptotic pattern with cells first becoming crenated, followed by nuclear condensation and DNA fragmentation.

The amount of antibody utilized did not result in cell proliferation or division and is thus ***submitogenic***.

Claim Rejections Under 35 U.S.C. §112, first paragraph

Claims 1 and 32-46 have been rejected under 35 U.S.C§112, first paragraph as not reasonably providing enablement for the instant claims. More specifically, the Examiner states:

> It is concluded in light of numerous conflicting teachings in patents cited foregoing, the invention does not appear to be enabled absence of clarification of the contradictory evidence found in the references.

Applicants traverse and submit that the teachings in the specification and the cited patents are not conflicting, and present the following clarification for the benefit of the Examiner.

The Examiner is concerned with the seemingly contradictory role of anti-CD3 antibodies in inducing or preventing programmed cell death of T cells, as reported in the art. The Examiner relies upon the disclosures of Nakai, *et al.* (US Patent No. 5,691,341) Kwon, *et al.* (US Patent No. 6,303,121) Lenardo (US Patent No 6,083,503), and Example 6 of the instant application, as teaching that contacting anti-CD3 antibodies to a T cell induces programmed cell death. In response, Applicants submit that the seemingly contradictory effects of anti-CD3 antibodies on T cells cited by the Examiner is actually related to the type of T cell which is contacted. Applicants' claims specifically recite that the T cell is *ex vivo*. Furthermore, claims 1 and 39 have been amended to specify that the T cell is a "mature" T cell.

The disclosure of Nakai, *et al.* refers to the phenomena known as activation-induced T cell death (AICD), known in the art to occur in ***immature T cells*** and also in some ***transformed*** cell lines and ***T cell hybridomas.*** The teaching of Nakai, *et al.* that anti-CD3 induces programmed cell death in T cells is specific to ***immature T cells and hybridomas***. AICD does not result from contact of a mature, resting T cell obtained from an individual with anti-CD3 antibodies. Evidence of this is found in the articles cited by Nakai, *et al.* in support of the statements (quoted by the Examiner) that apoptosis is inducible by anti-CD3 antibodies. Specifically, Smith, *et al.* (*Nature 337*: 181-184 (1989), submitted herewith) and Tadakuma, *et al.* (*Eur. J. Immunol. 20*: 279 (1990), submitted herewith), both teach that anti-CD3 antibodies induce ***mature*** T cells to proliferate, but induce ***immature*** thymocytes to die via apoptosis. Similarly, the disclosure of Kwon, *et al.* also refers to AICD as occurring in immature T cells, hybridomas and transformed cell lines. Further to this distinction, Example 6 of the instant application describes experiments performed on Jurkat cells, which are of a ***transformed cell line*** and subject to AICD. In contrast, Examples 1-5 utilize primary, mature T cells obtained from donor individuals, which are not subject to AICD.

The claims, as amended, which recite that the T cell is ***ex vivo***, and is a ***mature T cell***, clearly exclude immature T cells, transformed T cells and hybridomas, which are reported to undergo AICD upon exposure to anti-CD3 antibodies. Applicants therefore submit that the claims as amended may be performed by of one of ordinary skill in the art with no more than routine experimentation.

The Examiner further cites the disclosure of Kwon, *et al.* as teaching that "continued presence of anti-CD3 in cell culture would cause T cell unresponsiveness to even saturated anti-CD28 [levels]" resulting in cell death. Applicants submit that reactivation multiple times taught by Kwon, *et al.* is not equivalent to continued presence of anti-CD3 as recited by the Examiner. Applicants submit that the disclosure of Kwon, *et al.* teaches that activated T cells which are ***repeatedly reactivated*** (*e.g.,* three times) with ***high doses*** (*e.g.,* 10μg/ml) of anti-CD3 can become resistant to the effects of CD28, and anti-CD28. However, in an effort to expedite prosecution, Applicants have amended the instant claims to recite "***submitogenic*** amounts of anti-CD3". The teachings of Kwon, *et al.*, with respect to anti-CD3 inducing apoptosis, are specific to ***high doses*** of

anti-CD3 and thus methods for the use of the submitogenic amounts of anti-CD3 antibodies to protect a T cell from programmed cell death are clearly enabled.

The Examiner further suggests that the claims are not enabled with respect to cells previously exposed to IL-2, in light of the teachings of Lenardo that IL-2 causes T cells to undergo apoptosis upon re-immunization with an antigen. Applicants submit that the teachings of Lenardo do not indicate that the instant claims will not work. Lenardo teaches a specific effect of IL-2 exposure of T cells *in vitro* prior to antigen exposure, wherein subsequent re-exposure of the T cells to antigen within 2-3 days *in vitro* leads to T cell death. Lenardo further teaches that "the effects of IL-2 wear off 2-3 days after IL-2 is no longer present, hence rechallenge must occur within that period" (column 12, line 27-29). Since the effects of IL-2 exposure wear off relatively quickly, given the guidance of the specification and the knowledge of one skilled in the art, one so skilled could practice the claimed methods without inadvertently inducing cell death in cells which may have been previously exposed to IL-2.

With respect to superantigens, the Examiner argues that some superantigens have been observed to cause apoptosis, rather than protect T cells from apoptosis, as in the claimed method, citing Johnson, *et al.*, Lenardo, and Lynch, *et al.* Johnson, *et al.* simply reference a research article by Gougeon, M-L., L. Montagnier (1993) Science 260:1269-1270 in the Background section in which this group purportedly showed that superantigens may induce apoptosis. Applicants submit that the cited teachings of Johnson, *et al.* as they relate to *nef* as a superantigen, are highly speculative, and are not supported by experimental evidence. Although *nef* may be critical for HIV infection, the mechanism by which it acts is still unknown and more recent data suggests that "Nef" may act to prevent presentation of viral antigens in the context of the major histocompatibility complex or play a role in modulation of cellular signaling pathways but not that it functions as a superantigen, *e.g.*, "powerful T-cell mitogens that bind directly to major histocompatibility complex (MHC) class II molecules and form a binary complex with the variable .beta. (V.sub..beta.) region of the T-cell antigen receptor (TCR)". With respect to the teachings of Lenardo, that bacterial *Staphylococcus* superantigen induced T cell PCD in mice, and the teaching of Lynch, *et al.*, that certain antigens and superantigens cause T cell deletion in LPR mice, Applicants point out that the reported phenomena occur *in vivo*. No evidence is presented that apoptosis would

occur as a result of contact of the superantigens under *ex vivo* conditions as claimed. Applicants have presented evidence that a variety of superantigens can protect a mature T cell from cell death when utilized in the claimed methods, and have enabled a representative number of species for the claimed genus. Moreover, the determination of additional superantigens appropriate for use in the claimed methods is within the ability of one of ordinary skill in the art through no more than routine experimentation.

The Examiner further argues that polyclonal activators such as lectins are not enabled by the specification for use in the claimed methods, citing the teachings of Kwon, *et al.* as teaching the use of "PHA as a trigger to induce PCD in T cells." In response, Applicants argue that Kwon, *et al.* teaches activation of T cells with the lectin phytohemagluttinin, followed by induction of apoptosis of the activated T cells by ***repeated activation*** by anti-CD3 and anti-CD28 (column 18, lines 18-20). These teachings do not indicate that Applicants' claims are not enabled. The Examiner also cites the disclosure of Schlossman, *et al.* as teaching "treatment with anti-CD3 antibody, ionomycin, and/or phorbol ester can induce apoptosis in both human and mouse ***immature thymocytes***" and furthermore "culturing PBT cells with PMA-treated monocytes in medium containing ionoycin or PMA increased the level of apoptosis by almost 3- and 6- fold, respectively." Applicants submit that Schlossman, *et al.* teach a monocyte dependent activation of apoptosis in T cells, wherein the monocyte (or antigen presenting cells) are primed by exposure to phorbol myristate acetate or phytohaemagglutinin. Schlossman, *et al.* further teach that phytohaemagglutinin may be used to activate antigen presenting cells to induce their ability to prime resting T cells for apoptosis (column 4, line 24-32 and claim 5). Again, these teachings do not indicate that Applicants' claims are not enabled. It is within the ability of one of ordinary skill in the art to practice the claimed invention given the guidance presented in the application and the knowledge of one skilled in the art.

Regarding claims 39-46, the Examiner indicates that the claims will be evaluated by the standard of *in vivo* application, and states:

> the art is still unpredictable with regard to particular cell types and agents that enhance Bcl-X_L levels. ...many other cell surface receptors, apoptotic associated molecules, environmental factors also play a role in the state of the T cells, the specification fails to teach with regard to *in vivo* aspects of the invention, the influence of other factors, the disease and the disorder that would need T cell

protection, whether and how long the protective effect of in vitro T cell treatment would last. In view of such, the fact of treated T cells is highly unpredictable in a complicated *in vivo* environment, thus resulting in a trial and error situation.

Applicants submit that the art is not as unpredictable as the Examiner suggests and that the Examiner's reliance on certain teachings is misplaced. For instance, the disclosure of Boise, *et al.* is cited by the Examiner as teaching that "six hours of stimulation with PMA and ionomycin had no effect on bcl-x mRNA expression in double-positive thymocyte populations but induced a dramatic increase in bcl-x mRNA expression in both single-positive thymocytes and peripheral blood T cells." Applicants argue that these teachings ***support*** enablement of the present claims, as amended, which specify ***mature***, *e.g.,* single-positive, T cells, whereas double positive thymocytes, immature T cells, taught by Boise, *et al.* are unaffected by PMA and ionomycin. Thus, the findings of Boise, *et al.* are consistent with the claimed methods.

The Examiner further cites the disclosure of Gottschalk, *et al.* as teaching that "cyclosporin A, FK-506, and rapamycin could prevent PCD in T-cell hybridomas and thymocytes, but induce PCD in B cells," in support of the argument regarding the influence of other factors on the treated T cell. With respect to the teachings of Gottschalk, *et al.*, Applicants point out that these teachings are not contradictive to the effectiveness of the claimed methods since the claims are directed to methods for protecting ***T cells*** from apoptosis. The teachings of Gottschalk, *et al.*, as per the comments of the Examiner that "many other cell surface receptors, apoptotic associated molecules, environmental factors also play a role in the state of the T cells," have no bearing on the instant claims.

The Examiner further cites the disclosure of Roberts as teaching the involvement of other cell surface receptors, apoptotic associated molecules, and environmental factors playing a role in the state of T cells. The disclosure of Roberts teaches chimeric receptors used to genetically engineer immune cells to respond to costimulation through chimeric receptors, *e.g.*, to prevent the induction of anergy in the recipient cells. These teachings have no bearing on the instant claims.

Regarding the Examiner's position that the specification fails to teach the disease and the disorder that would need T cell protection, Applicants submit that the ordinary

skilled artisan would, at the priority date of the instant application, understand that the claimed methods would be useful in the treatment of an individual in need of an increased immune response, *e.g.*, individuals infected with pathogens which cause T cell death, or for boosting an immune reaction in order to more rapidly eliminate an infection (page 32, line 9-10). Applicants direct the Examiner's attention to page 31, line 1-3 of the specification, which indicates that the methods of the present invention are useful for preventing cell death of CD4+ T cells of an HIV infected individual and protecting T cells from HIV infection. Further to this, Example 9 of the application details experiments that indicate bcl-X_L expression can prevent HIV-1-induced cell death. The specification also teaches that the methods of the invention are useful for treating T cell associated disorders, or those disorders associated with T cells having an extended lifespan, including but not limited to autoimmune diseases and disorders and graft versus host disease (page 32, line 30).

Regarding the concerns of the Examiner's concerns surrounding the duration of the protection conferred to the T cell upon reintroduction into the subject, Applicants again argue that the Examiner has failed to provide teachings in the art that indicate that the duration conferred would be insufficient for useful treatment, and further argue that even a short lived effect of apoptosis resistance of the T cell could provide beneficial results to an individual. Moreover, if necessary the treatment could be repeated to produce optimal effects. In addition, under certain circumstances, a short lived duration would be preferred (see for example, page 32, line 20-23).

In summary, Applicants have amended the claims to clearly indicate that the invention is drawn to the use of "mature T cells", and also to recite that "submitogenic amounts of anti-CD3 antibody" are used. The application clearly teaches and enables the presently claimed methods. Applicants therefore respectfully request reconsideration and withdrawal of the rejection.

Claim Rejection Under 35 U.S.C. §102

Claim 1 has been rejected under 35 U.S.C. 102(e) as being anticipated by US Patent 6,352,694. Applicants note that this patent is referred to by the Examiner as "Thompson, *et al.*," however in fact the inventors listed on this patent are actually June,

et al. This response is therefore made with the assumption that the Examiner meant to cite June, *et al.*, US Patent No. 6,352,694. The Examiner describes the teachings of that June, *et al.* as follows:

> "a method comprising contacting T cells *in vitro* with an anti-CD3 antibody (step a of claims 1 and 17 of the cited patent), then an anti-CD28 antibody (step b of claim 1 of the cited patent) or a CD28 ligand selected from B7-1 or B7-2 (step b of claim 17)" and also that "T cells could be used in treating infectious disease and cancer, which embrace HIV (claims 15, 16, 31, and 32)."

In response, Applicants point out that protection from apoptosis is not necessarily coincident with induction of cell proliferation, and likewise, a culture which is protected from apoptosis, is not necessarily proliferating. Indeed, a proliferating cell culture may still be undergoing a significant amount of apoptosis. Applicants have identified a function of anti-CD3 in T cells which is independent of its proliferative activity. Claims 1 and 39 as amended specify a ***submitogenic*** of amount of anti-CD3 antibody which is not taught or suggested by June, *et al.* Applicants therefore request withdrawal of the rejection.

Claim Rejection Under 35 U.S.C. §103

Applicants gratefully acknowledge the Examiner's indication in the Advisory Action dated March 10, 2003 that Applicant's Amendment After Final response overcomes the rejection of claims 1, 34, and 38 under 35 U.S.C. §103(a) as being unpatentable over Lenardo in view of Roberts. Since this amendment will now be entered, Applicants request withdrawal of this rejection.

Double Patenting

Claims 1, 34, 37, and 38 have been rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over claims 1, 15-19, 31, and 32 of U.S. Patent No. 6,352,694 (June, *et al.*). More specifically the Examiner states:

> Although the conflicting claims are not identical, they are not patentably distinct from each other because claims 1, 34, 37, and 38 of the present application and claims 1, 15-19, 31, and 32 of the cited patent are each drawn to a method

comprising the steps of contacting the T cell with agents, i.e. anti-CD3 antibody, and anti-CD28 antibody, or a CD28 ligand, wherein the T cells are HIV infected (infectious and cancerous).

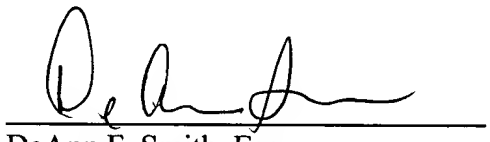
The processes of the present application and the cited patent differ from the other in the preamble recitations, however, the recitations "for inducing a population of T cell" in the cited patent or "for protecting a T cell from cell death" in the present application are obvious variants, *i.e.* T cells in a healthy growing state are resistant to apoptosis.

In response to the above quoted comments by the Examiner, Applicants reiterate the above stated argument that a proliferative population of cells is not necessarily resistant to apoptosis. Moreover, in an effort to expedite prosecution, claims 1 and 39 have been amended to specifically recite a ***submitogenic amount*** of anti-CD3. Applicants thus submit that the currently pending claims are patentable over June, *et al.* and request withdrawal of this rejection.

SUMMARY

In view of the amendments and remarks set forth above, it is respectfully submitted that this application is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with Applicants' Attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at (617) 227-7400.

Respectfully submitted,



DeAnn F. Smith, Esq.
Registration No. 36,683
Agent for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
(617) 227-5941

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